

**MIMULUS SOOKENSIS (PHRYMACEAE), A NEW ALLOTETRAPLOID SPECIES
DERIVED FROM *MIMULUS GUTTATUS* AND *MIMULUS NASUTUS***

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ABSTRACT

A new species of monkeyflower, *Mimulus sookensis*, is described. This species is found throughout the southern portion of Vancouver Island, the Gulf Islands of British Columbia, the San Juan Islands of Washington state, the Willamette and Umpqua River Valleys in Oregon, and has been collected at one location in Mendocino County, California. *Mimulus sookensis* is a tetraploid species ($n = 28$) derived from the predominately outcrossing *Mimulus guttatus* DC. ($n = 14$) and the predominately self-pollinating *Mimulus nasutus* Greene ($n = 14$). *Mimulus sookensis* is similar phenotypically to the small-flowered *M. nasutus*, but differs in chromosome number, height, and by a slightly more narrowed corolla tube than that of *M. nasutus*. It is commonly found on wet hillsides, seeps, cutbanks, and in roadside ditches, often co-occurring with *M. guttatus* but infrequently with *M. nasutus*.

Key Words: Allotetraploid speciation, *Mimulus*, *Mimulus guttatus*, *Mimulus nasutus*, monkeyflower, new species, Oregon, Vancouver Island.

A small-flowered monkeyflower similar to *Mimulus nasutus* Greene was first observed on Vancouver Island, Canada, by Fred Ganders, and later collected for scientific study in May 1991 by Beverly Benedict. Although phenotypically similar to *M. nasutus* (Fig. 1), allozyme analysis revealed that some of the small-flowered monkeyflowers on Vancouver Island were always heterozygous at allozyme markers. This was in contrast to allozyme data from another small-flowered monkeyflower found on the island, *M. nasutus* (snouted monkeyflower), and the common yellow monkeyflower, *M. guttatus* DC. These results were intriguing because while the large-flowered, chasmogamous *M. guttatus* is known to be highly outcrossing, both *M. nasutus* and the

heterozygous, small-flowered monkeyflowers were known to be highly selfing, given their floral structure, small flower size, and often cleistogamous nature (Ritland and Ritland 1989; Dole 1992; Willis 1993). Morphological analysis of *M. guttatus*, and the two small-flowered monkeyflowers (*M. nasutus* and the species described here, *M. sookensis*) revealed that while *M. nasutus* and *M. sookensis* overlapped a great deal in floral morphology, subtle morphological differences did exist (Fig. 1, e.g., pistil length, corolla tube width). Because of fixed heterozygosity in some of the small-flowered *Mimulus* on Vancouver Island, and slight differences in floral morphology, F. Ganders suspected that the heterozygous monkeyflowers in question were actually a distinct taxon of allopolyploid origin (Benedict 1993). Chromosome squashes conducted at the time revealed that these new monkeyflowers, *M.*

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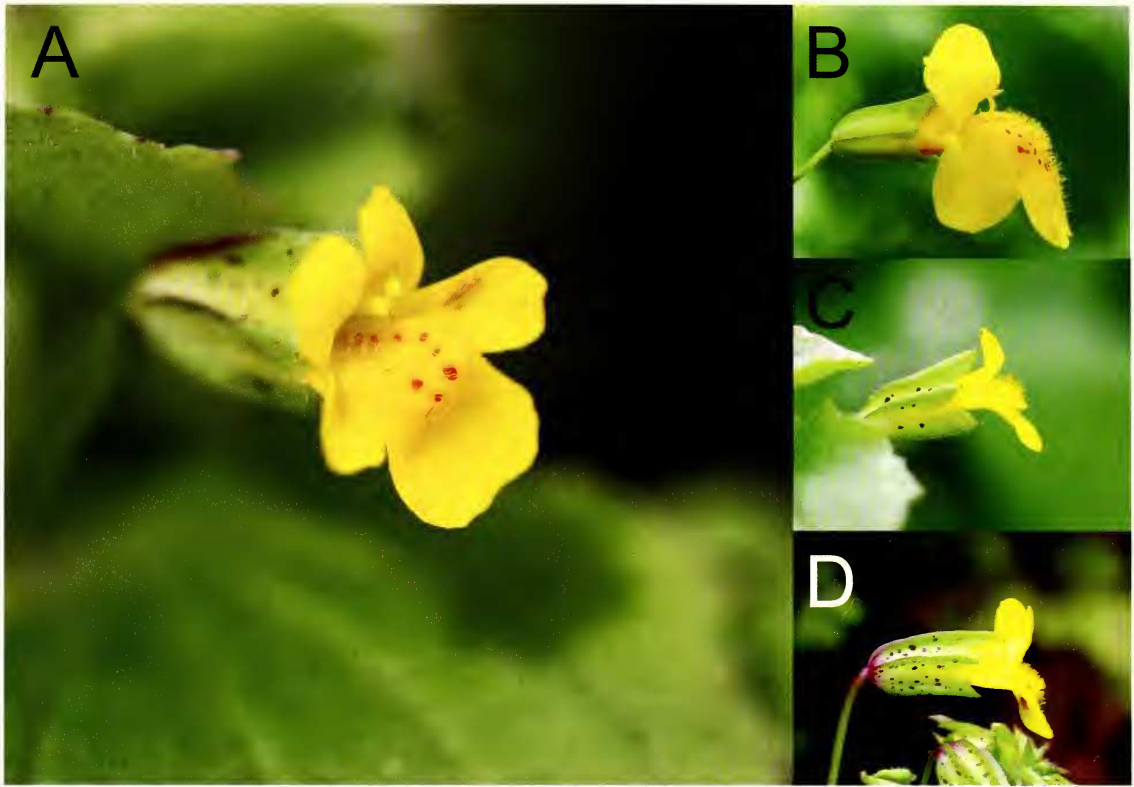


FIG. 1. Photographs of *M. sookensis* and its progenitor species. Side profile photographs are taken on approximately the same scale. A. *M. sookensis*, B. *M. guttatus*, C. *M. sookensis*, D. *M. nasutus*.

sookensis, had more than $n = 14$ chromosomes, but an exact count was not obtained.

Following the work of B. G. Benedict, flow cytometry data from three *M. sookensis* collections revealed that the small-flowered monkey-flowers from Vancouver Island and surrounding areas, as well as the valleys of western Oregon and northern California, had approximately twice the DNA content of *M. guttatus* and *M. nasutus*, suggesting again that this taxon was of tetraploid origin (Sweigart et al. 2008). Sequence data from two nuclear genes confirmed that this new species was a hybrid tetraploid derived from *M. guttatus* and *M. nasutus*. Furthermore, crossing data revealed that the allotetraploids were reproductively isolated from their diploid progenitors due to failure of seed development, a result consistent with the triploid block that is commonly observed in interploidy crosses (Sweigart et al. 2008). Although *M. sookensis* is a cryptic species due to its phenotypic similarity to *M. nasutus*, the fact that it is reproductively isolated from its diploid progenitors illustrates the concept of instant or rapid speciation of polyploids, which has long been recognized (e.g., Winge 1917; Dobzhansky 1937; Coyne and Orr 2004). Polyploidy not only has the propensity to quickly create new species (according to the biological

species concept, e.g., Mayr 1996) but has contributed significantly to angiosperm evolution (Stebbins 1971; Grant 1981; Masterson 1994; Otto and Whitton 2000).

Here, we present evidence that *M. sookensis* is historically taxonomically unrecognized, and provide new chromosome data that provide conclusive evidence that *M. sookensis* is a cytologically distinct species, which has previously been shown (Sweigart et al. 2008) to be of polyploid origin, and reproductively isolated from its diploid progenitors, as well as a description of this hitherto unnamed species of monkeyflower.

REVIEW OF PREVIOUSLY PUBLISHED
MIMULUS TAXA

Mimulus guttatus is an herbaceous wildflower distributed throughout much of western North America (Vickery 1978), while *Mimulus nasutus* has a restricted range relative to *M. guttatus* (Kiang and Hamrick 1978; Vickery 1978). *Mimulus guttatus*, *M. nasutus*, and *M. sookensis* all belong to the *M. guttatus* species complex, and are part of the Simiolus clade (Beardsley et al. 2004) of the genus *Mimulus*. *Mimulus guttatus* and its close relatives have been extensively

TABLE 1. COMPLETE LIST OF PREVIOUSLY PUBLISHED *MIMULUS* TAXA WHICH MIGHT HAVE BEEN A DESCRIPTION OF *M. SOOKENSIS*, WITH A DESCRIPTION OF HOW THEY ARE DIFFERENT FROM *M. SOOKENSIS*. Taxa are listed in alphabetical order, although subspecies and varieties are listed in parentheses if variety or subspecies was given specific rank. For synonyms examined, three sources were used: the synonyms listed in Grant (1924) and Pennell (1951) for *M. nasutus*, and the synonyms listed for both *M. guttatus* and *M. nasutus* in IPNI. Many of the large flowered varieties of *M. guttatus* were not included in this list for the purpose of brevity. All references are included in the literature cited. Evidence sources refers to all herbarium specimens, drawings and descriptions, in both the nomenclatural citation and established floras or monographs, that were used in determining differences. For each candidate taxa, the characters that most easily illustrate the difference between the listed taxa and *M. sookensis* are described for the listed taxa.

Previously published taxa	Synonym (source)	Evidence sources	Distinguishing characters from <i>M. sookensis</i>
<i>M. arvensis</i> Greene (<i>M. guttatus</i> DC. var. <i>arvensis</i> Grant)	<i>M. guttatus</i> (IPNI)	Greene (1887); Grant (1924); Pennell (1951); Mukherjee and Vickery (1962)	Diploid ($n = 14$), easily hybridizes with <i>M. guttatus</i> ; Greene describes the leaves as lyrate, and the species as perhaps synonymous with <i>M. lyratus</i> Benth. Grant describes the variety as having an upper calyx tooth not markedly longer than others, elongated internodes, teeth not usually folded over each other at maturity
<i>M. bakeri</i> Gandoger	<i>M. nasutus</i> (Grant 1924, Pennell 1951)	CAS 22488 (isotype), NY 20798 (possible isotype); Gandoger (1919)	CAS specimen appears to be hybrid between <i>M. guttatus</i> and <i>M. nasutus</i> , while description doesn't match specimen, description suggests that difference between <i>M. nasutus</i> and <i>M. bakeri</i> is the impunctate calyx of <i>M. bakeri</i>
<i>M. cordatus</i> Greene	<i>M. nasutus</i> (Grant 1924)	Greene (1910); Pennell (1951); Mukherjee and Vickery (1962)	Corolla lacking in spotting, diploid ($n = 14$) that hybridizes easily with <i>M. guttatus</i>
<i>M. cuspidatus</i> Greene	<i>M. nasutus</i> (Grant 1924; Pennell 1951)	DS 771002 (isotype); description in Greene (1910)	<i>M. nasutus</i> found in wet shades exhibiting phenotypic plasticity in a classic shade avoidance response (see text for discussion)
<i>M. decorus</i> (Grant) Suksd. (<i>M. guttatus</i> DC. var. <i>decorus</i> Grant)	<i>M. guttatus</i> (IPNI)	CAS 22445; Pennell (1951)	Corolla large
<i>M. erosus</i> Greene	<i>M. nasutus</i> (Grant 1924)	Greene (1910)	Corolla exerted from tube, synonym of <i>M. nasutus</i>
<i>M. glareosus</i> Greene	<i>M. nasutus</i> (Grant 1924; Pennell 1951)	Greene (1889, 1894)	Leaves toothed or lobed, slimy, synonym of <i>M. nasutus</i>
<i>M. guttatus</i> DC. subsp. <i>scouleri</i> (Hook.) Pennell	<i>M. guttatus</i> (IPNI)	Pennell (1947)	Stoloniferous variant of <i>M. guttatus</i> with more linear leaves (perhaps synonymous with <i>M. tilingii</i> Regel or <i>M. caespitosus</i> Greene)
<i>M. hallii</i> Greene (<i>M. guttatus</i> DC. var. <i>hallii</i> Grant)	<i>M. guttatus</i> (IPNI)	Greene (1885); Grant (1924)	Leaves parallel-veined and almost entire, calyx highly inflated
<i>M. guttatus</i> DC. var. <i>lyratus</i> (Benth.) Pennell ex M. Peck	<i>M. guttatus</i> (IPNI)	Pennell (1941); Pennell (1951)	Leaves pinnately lobed at the base, corolla long (2–3 cm)
<i>M. guttatus</i> var. <i>depauperatus</i> Grant (<i>M. luteus</i> var. <i>depauperatus</i> A. Gray)	<i>M. guttatus</i> (IPNI)	Gray (1867); Grant (1924); Hitchcock and Cronquist (1987)	Grant (1924) thought to be synonymous with <i>M. puncticalyx</i> and <i>M. microphyllus</i> , based on Hitchcock and Cronquist (1987) and Gray, appears to be simply a description of small <i>M. guttatus</i> or <i>M. nasutus</i> plants with few or small flowers – a condition most likely caused by environment
<i>M. guttatus</i> var. <i>glaucescens</i> (Greene) Jeps. (<i>M. glaucescens</i>)	<i>M. guttatus</i> (IPNI)	Greene (1885); Jepson (1925); Pennell (1951)	Leaves glaucous, synonymous with <i>M. glaucescens</i> (Greene)

TABLE 1. CONTINUED.

Previously published taxa	Synonym (source)	Evidence sources	Distinguishing characters from <i>M. sookensis</i>
<i>M. guttatus</i> var. <i>gracilis</i> (A. Gray ex Torr.) Campbell	<i>M. guttatus</i> (IPNI)	CAS 23523 (isotype for <i>M. pardalis</i>); Campbell (1950); ORE96554	Campbell lumps all synonyms of <i>M. nasutus</i> and <i>M. nasutus</i> itself under this variety. CAS specimen is <i>M. pardalis</i> , corolla described as being twice as long as the calyx, diploid ($n = 14$)
<i>M. guttatus</i> var. <i>nasutus</i> Jeps.	<i>M. nasutus</i> (Pennell 1951)		Synonym of <i>M. nasutus</i>
<i>M. guttatus</i> var. <i>puberulus</i> A. L. Grant	<i>M. guttatus</i> (IPNI)	Grant (1924)	Listed as perennial, large-flowered
<i>M. inflatulus</i> Suksd.	<i>M. breviflorus</i> (Pennell 1951)	CAS 152750 (isolectotype); Pennell (1951)	Calyx equal-toothed, leaves more linear and narrow, synonym of <i>M. breviflorus</i> Piper
<i>M. laxus</i> Pennell ex. M. Peck	<i>M. guttatus</i> (Mukherjee and Vickery 1962)	CAS 329746 (isotype); NY 90734 (isotype); Mukherjee and Vickery (1962)	Variant of <i>M. guttatus</i> , diploid ($n = 14$)
<i>M. marmoratus</i> Greene	<i>M. nasutus</i> (Pennell 1951)	Greene (1895b)	Description of <i>M. marmoratus</i> matches that of a hybrid between <i>M. guttatus</i> and <i>M. nasutus</i> , with large red blotch on middle lower lobe, with a corolla that is longer than <i>M. nasutus</i> (>3 cm)
<i>M. micranthus</i> A. Heller (<i>M. guttatus</i> var. <i>micranthus</i> (A. Heller) G. R. Campb., <i>M. nasutus</i> Greene var. <i>micranthus</i> A. L. Grant)	<i>M. guttatus</i> , <i>M. nasutus</i> (IPNI)	DS 74105; NY 90746 (isotype) Heller 7410; Heller (1912); Grant (1924); Pennell (1951); Munz (1959)	Narrow-range endemic of CA; calyx even toothed and lower teeth not curled upward and inward upon maturity, stem weak, lower leaves described as being lyrate and long-petioled, calyx puberulent, diploid used in multiple genetic studies (see text)
<i>M. microphyllus</i> Benth. (<i>M. guttatus</i> var. <i>microphyllus</i> Pennell in M. Peck)		Greene (1885), Pennell (1941, 1951)	Leaves small, stems rounded, pistil much exserted from calyx, located mostly in the mountains
<i>M. minusculus</i> Greene	<i>M. nasutus</i> (Grant 1924)	Greene (1910)	Perennial, shorter than <i>M. sookensis</i> , leaves ovate, flowers large
<i>M. minutiflorus</i> R. K. Vickery		CAS 961575 (isotype); Vickery (1997)	Corolla superficially similar in appearance to <i>M. sookensis</i> , but lacking ridges, and stems wiry; closely related to <i>M. wiensii</i> , $n = 32$
<i>M. nasutus</i> Greene var. <i>eximius</i> Green A. L. Grant ex J. T. Howell	<i>M. nasutus</i> (IPNI)	Howell (1949)	Howell (1949) bases his description of this variety on <i>M. nasutus</i> , but does not realize that what he considers <i>M. nasutus</i> is actually a hybrid between <i>M. guttatus</i> and <i>M. nasutus</i> , also appears to be synonymous with <i>M. nasutus</i> var. <i>insignis</i>
<i>M. nasutus</i> Greene var. <i>insignis</i> A. L. Grant	<i>M. nasutus</i> (IPNI)	Grant (1924); and Pennell (1941)	Flower size outside the range of <i>M. sookensis</i> and large blotch of anthocyanin spotting on lower corolla lobe, both suggest that description matches that of a hybrid between <i>M. guttatus</i> and <i>M. nasutus</i>
<i>M. guttatus</i> DC. var. <i>insignis</i> Greene	<i>M. guttatus</i> (IPNI)	JEPS 2938 (the very type!)	Large flowered, hybrid between <i>M. guttatus</i> and <i>M. nasutus</i>
<i>M. parishii</i> Gand.	<i>M. nasutus</i> (Grant 1924; Pennell 1951)	Gandoger (1919)	Leaves deeply cut or laciniate; only a single specimen was examined in the naming
<i>M. puberulus</i> Greene		Greene (1910)	Corolla large (>3 cm), stem round and viscidly puberulent
<i>M. puberulus</i> Gand.	<i>M. nasutus</i> (Grant 1924; Pennell 1951)	Gandoger (1919)	Only distinguishing feature from typical <i>M. nasutus</i> is that it is minutely pubescent; only a single specimen was examined, a synonym of <i>M. nasutus</i>

TABLE 1. CONTINUED.

Previously published taxa	Synonym (source)	Evidence sources	Distinguishing characters from <i>M. sookensis</i>
<i>M. puncticalyx</i> Gand.	<i>M. nasutus</i> (Pennell 1951)	ORE96654 (isotype); ORE96655; Gandoger (1919)	Leaves tiny upper tooth hardly more prominent than others; only a single specimen was examined in the naming
<i>M. subreniformis</i> Greene	<i>M. nasutus</i> (Grant 1924; Pennell 1951)	UC 27111 (holotype); Greene (1895a)	Appears to be a diminutive variant of <i>M. nasutus</i> , but without anthocyanin spotting on corolla
<i>M. washingtonensis</i> Gand.		CAS 152669 (isotype); Gandoger (1919)	Calyx equal-toothed, flowers large

collected and examined throughout western North America, by both early botanists and contemporary botanists and geneticists. Historically, *M. guttatus* and its close relatives have been subject to extraordinarily divergent taxonomic treatments by different authors. Pennell (1951) recognized 28 taxa closely allied with *M. guttatus* from the Pacific Northwest, and in a recent treatment of California, Thompson (1993) recognized only five. In contemporary times, the genus *Mimulus* has seen a proliferation of scientific interest: a Google Scholar search for articles published between 1980–2011 including the word *Mimulus* in the title found 436 articles, with 194 written on *M. guttatus* alone. Although many of these recent publications do not necessarily include field work, it is safe to assert that more has been learned of the genetics, ecology, distribution, and taxonomic status of *M. guttatus* and its close relatives, since the publications of Grant (1924), Pennell (1951) and even Thompson (1993), see Wu et al. (2008). By combining knowledge from contemporary studies with historical taxonomic wisdom, we found that **M. sookensis** is truly a previously overlooked species in this intensely studied group, in part due to its cryptic nature.

To determine if **M. sookensis** was previously taxonomically recognized, we first identified synonyms of *M. guttatus* (only the small-flowered or obscure taxa) and *M. nasutus*, from those listed in Pennell (1951), Grant (1924), and Campbell (1950), and from lists of synonyms derived from IPNI (International Plant Names Index). We also searched in Pennell (1951) and Grant (1924) for descriptions of small, yellow-flower *Mimulus* that were not listed as synonyms of *M. guttatus* or *M. nasutus*, but were considered to be closely related to the Simiolus clade (candidate taxa, Table 1). For these 31 candidate taxa, in which the author might have potentially described **M. sookensis**, we referred to herbarium specimens, the original species descriptions, crossing data and chromosome counts (when

available), and drawings and descriptions in other references to determine if a previously published name could be applied to **M. sookensis** (Table 1). We did not find a previously published taxon that satisfied every aspect of the morphology and cytology of **M. sookensis** (Table 1), and thus, despite the abundance of synonyms within the *M. guttatus* species complex, no previously published names can be applied to **M. sookensis**.

Throughout the course of our examination of **M. sookensis** candidates, we found that the reasons why candidate taxa were not representative of **M. sookensis** fell into one or more categories. First, pronounced differences in habit, leaf, and even floral morphology existed (e.g., perenniality, lyrate leaves, even-toothed calyx). Second, in some cases the species described was likely either a hybrid between *M. guttatus* and *M. nasutus*, or *M. nasutus*. In the field, *M. guttatus* and *M. nasutus* are known to hybridize when they co-occur (Kiang 1973; Martin and Willis 2007). Hybrids between *M. guttatus* and *M. nasutus* have flowers that are much more similar in size to *M. guttatus*, due to dominance of the *M. guttatus* floral genes (Fishman et al. 2002). In the field, a prominent red blotch has often been observed on the lower middle corolla lobe of both *M. nasutus* (e.g., Pennell 1951; Kiang 1973) and some monkeyflowers with larger flowers than those of typical *M. nasutus*, but bearing resemblance to *M. nasutus* in shoot architecture and leaf morphology. This prominent red blotch has not been observed on **M. sookensis** flowers. The fact that the species described often had both larger flowers and a large red blotch suggests that they are either *M. nasutus* or hybrids between *M. guttatus* and *M. nasutus*. Third, there were some cases in which floral morphology differences were subtle, but differences in chromosome number existed, based on crossing studies and chromosome counts of Vickery (Campbell 1950; Mukherjee and Vickery 1962). In the special case of *Mimulus micranthus* A. Heller, it is defined in part by its endemism (Munz 1959). *Mimulus*

TABLE 2. LIST OF COLLECTIONS USED IN MEIOTIC CHROMOSOME COUNTS AND IN THE PREVIOUSLY PUBLISHED FLOW CYTOMETRY ANALYSES PRESENTED IN SWEIGART ET AL. (2008). Abbreviations: MCC, meiotic chromosome count; FC, flow cyometry.

Collection	Taxon	Locale	Longitude	Latitude	Analyses
DRN (DEX)	<i>M. sookensis</i>	Dexter's Reservoir, OR, USA	-122.756	43.917	FC
LSN	<i>M. sookensis</i>	Lowell, OR, USA	-122.784	43.930	MCC, FC
NHI	<i>M. sookensis</i>	Nanoose Hill, VI, BC, CAN	-124.160	49.273	MCC
ROG	<i>M. sookensis</i>	ca. 12 mi SE of Marial, (as the crow flies) OR, USA	-123.644	42.657	MCC, FC
TRT	<i>M. nasutus</i>	near Troutdale, OR, USA	-122.368	45.520	MCC

micranthus is a diploid that has been used in multiple genetic analyses, and has been successfully crossed with other known diploids (Fenster and Ritland 1992, 1994; Ritland et al. 1993; Fenster et al. 1995). Last, we believe that the species described in some cases were possibly representative of phenotypic plasticity, the most notable being *M. cuspidatus* Greene, found growing in shaded spots, with elongated internodes, and lack of anthocyanin spotting. In *Impatiens capensis* Meerb., this phenotype is known to be an adaptive plastic response (Schmitt et al. 1995; Dixon et al. 2001) that is characteristic of the classic shade avoidance syndrome (Smith 1982; Smith and Whitelam 1997). While it is not possible to directly test for plasticity in previously collected specimens, it seems highly plausible that many of the candidate taxa that we examined are representative of either phenotypic variation or plasticity in *M. nasutus* or *M. guttatus*. Grant (1924) noted that *M. nasutus* appeared to be quite a plastic species, and thus the taxa's earlier designations (e.g., Grant 1924; Pennell 1951) as synonyms are appropriate. Additionally, Kiang (1973) demonstrated that *Mimulus nasutus* is an exceptionally plastic species, as the flower size is dependent upon both external environmental conditions, and the position of the flower along the stem. It is also well known that *M. guttatus* harbors a great deal of phenotypic variation (reviewed in Wu et al. 2008).

CYTOLOGICAL ANALYSIS

Meiotic counts of chromosomes were conducted to corroborate the previous indications of polyploidy as evidenced by flow cytometry (Table 2), crossing barriers, (Sweigart et al. 2008), and fixed heterozygosity at allozymes and sequenced nuclear loci (Benedict 1993; Sweigart et al. 2008). Three individuals, each from different collections considered to be **M. sookensis** (Table 2, LSN, NHI, ROG) were used for the chromosome counts. A single diploid *M. nasutus* individual (TRT) was also counted, for the purpose of comparing chromosome sizes. Immature flower buds were collected in a 3:1 95% ethanol:glacial acetic acid solution. The tissue

was transferred to 70% ethanol after 24 hr and stored at -20°C until ready for use. Flower buds were then partially dissected in a 70% ethanol solution. The partially-dissected floral material was then transferred to a half-strength aceto-carmin solution, where all non-anther material was removed. Anthers were then transferred to a drop of aceto-carmin on a slide, and were eviscerated to release the pollen mother cells from the anthers. After thorough evisceration, the tissue was removed from the solution, and the slide was placed on a warming plate to facilitate staining. A drop of Hoyer's solution (Anderson 1954) was then added and the chromosomes were squashed by placing a coverslip over the solution and pressing down. Stained cells were examined with brightfield microscopy at 630-1000× magnification using a Zeiss Axioplan 2 microscope, and photographed at 1000× with a mounted Axiovision HR camera.

Meiotic chromosome counts revealed 28 distinct chromosome pairs in **M. sookensis** and 14 distinct chromosome pairs in diploid *M. nasutus* (Fig. 2). Although the sister chromatids are not easily distinguishable, it is clear from the chromosome squashes that there are twice as many of the chromosomes in **M. sookensis** as there are in diploid *M. nasutus*. This chromosome count constitutes the first published count for **M. sookensis**. Using these chromosome numbers as a calibration, we were able to confirm that the specimens used in the flow cytometry analysis of Sweigart et al (2008, Table 2) were indeed allotetraploid.

TAXONOMIC TREATMENT

Mimulus sookensis B. G. Benedict, J. L. Modliszewski, A. L. Sweigart, N. H. Martin, F. R. Ganders, and J. H. Willis, sp. nov. —TYPE: CANADA, British Columbia, on a southwest facing, open, wet hillside in Sooke Potholes Provincial Park beside the Sooke River, elev. 75 m, 48°24'N 123°43' W, 1 May 1991, Benedict 28 V207976 (holotype: UBC).

Herba annua obligata, a *Mimulus guttatus* DC. Pistillo 5–13 mm longo, corolla 6–20 mm longa et pistillo calycem aequante vel paulo longiore

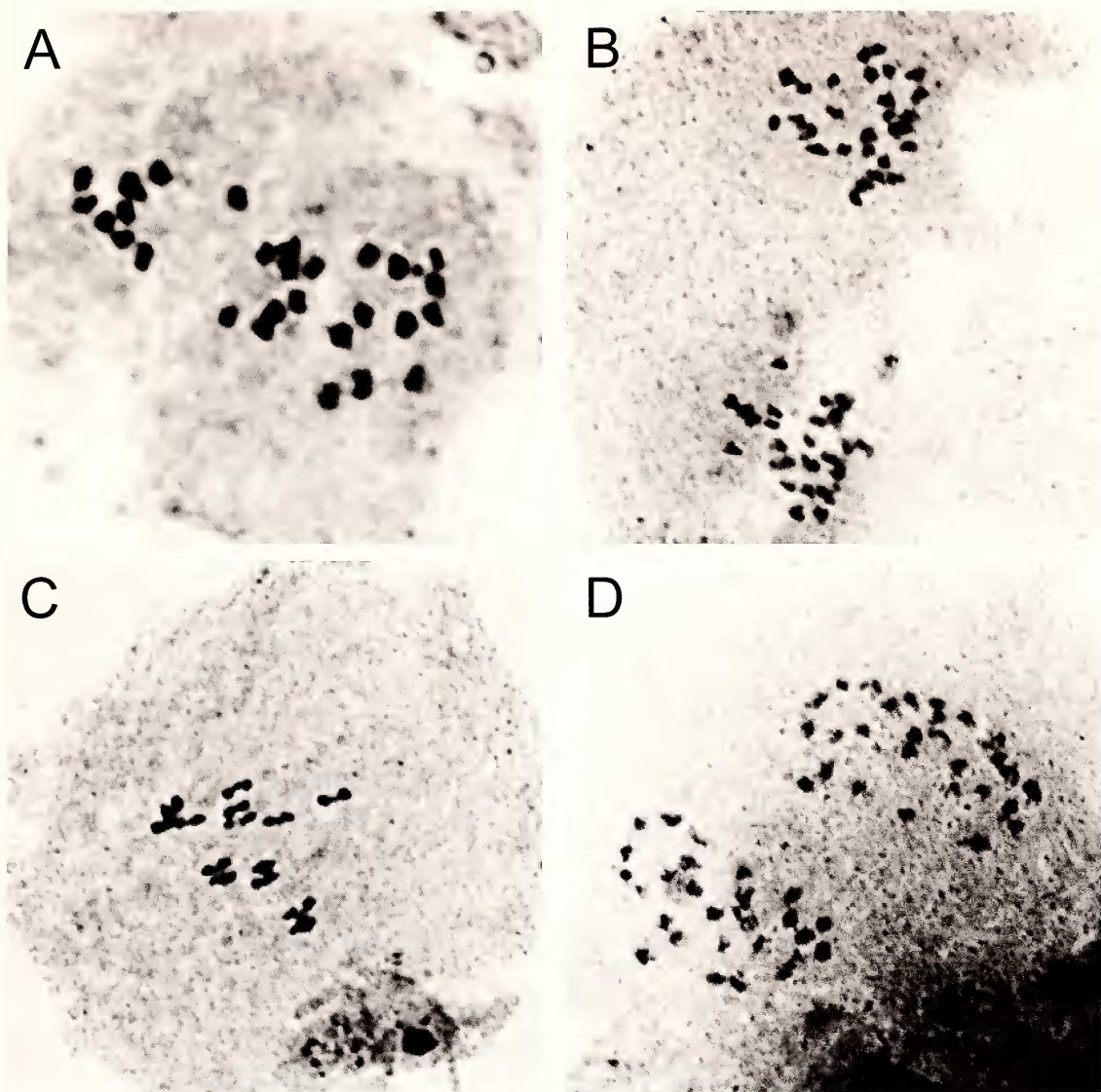


FIG. 2. Meiotic chromosome counts in *Mimulus*. A. *M. sookensis* (LSN), with 28 bivalents as seen in prophase I of meiosis. B. *M. sookensis* (NHI), shown with two daughter cells at late telophase I. Upper cell has 28 distinguishable univalents, while the lower cell has ca. 28 univalents. C. *M. nasutus* (TRT), with 14 bivalents at prophase I of meiosis. D. *M. sookensis* (ROG) as seen at late telophase I of meiosis, with two daughter cells each possessing 28 univalents.

differ; a foliis non bullatu, et caulis non alatis differ; planta tetraploidea.

Annual or winter annual herb, bearing opposite pedicellate basal leaves graduating into sessile cauline leaves, 5–25 cm high, glabrous to minutely pubescent. **Roots** fibrous. **Leaves** with leaf blade palmately veined, regularly denticulate, widely ovate, apex obtuse to acute, $0.5\text{--}3 \times 0.5\text{--}2.5$ cm becoming gradually reduced up the stem; leaf blade above adaxially green, frequently with anthocyanic spotting, glabrous to minutely pubescent, veins often purplish red near leafbase; leaf surface below abaxially silver-green to purple, glabrous, veins green. **Petiole** 0–2 cm

long, green-white to red-white; glabrous. **Stems** tending to quadrangular but not winged, <2 mm wide. **Inflorescence** few flowered to racemose, terminal, with 1 primary raceme, occasional secondary racemes arising from leaf axils, flowers opposite in leaf axils. **Pedicel** 3–22 mm long, red, glabrous. **Calyx** 5–13 mm long, central adaxial calyx lobe longer than other four, green, often with anthocyanic spotting, white hairs on margin, somewhat inflated upon maturity. **Corolla** bilabiate or sometimes cleistogamous, $5\text{--}22 \times 2\text{--}13$ mm, yellow, corolla lobes subequal, palate densely hairy, red spotted, extending into tubes as two ridges, tube narrowly funnel shaped,

4–13 mm long. **Stamens** didynamous, upper stamens shorter, long stamens 4–12 mm. **Pistil** 5–13 mm; style white, minutely pubescent; stigma yellow, usually slightly exerted from calyx; ovary 2–5 mm, green; stipe 0–1 mm; stigma lobes may be thigmotropic. **Capsule** dehiscent by longitudinal slits with persistent style, crowned by a persistent calyx; lower calyx lobes curved upwards toward upper calyx lobe upon maturity. **Seeds** up to 300 per capsule, oval, brown, 0.5×0.2 mm. **Chromosome number** tetraploid, $n = 28$.

Found on wet, sunny, hillsides, cutbanks, and ditches on Vancouver Island and the Gulf Islands, British Columbia, on the San Juan Islands of Washington state, in the Willamette and Umqua River Valleys in Oregon, and also in one known site in Dos Rios, Mendocino Co., California, from sea level to 600 m. Flowers from late March to May.

The species is named after Sooke Potholes Provincial Park on Vancouver Island where it was found to grow abundantly and where the type specimen was collected. The common name shy monkeyflower is suggested, because this monkeyflower disguises itself as *M. nasutus*, and the flowers are small, in contrast to the 'gay' and gregarious flowers of *M. guttatus* (Vickery 1952).

Additional *M. sookensis* Specimens Examined

CANADA. B.C.: Lasqueti Island, Trematon Mountain, 19 May 1985, *Ceska* 19167 (V 144698); N. Pender Island, Oak Bluffs, 4 Apr 1983, *Ceska and Olgilve* 14245 (V 133335); Saltspring Island, 5 1/2 km SW of Ganges, Lot 34, 18 April 1976, *Douglas* 9716 (V 136977); Saltspring Island, clearing at the end of Isabella Road, 18 May 1980, *Benedict* 3 (UBC 207936); Mayne Island, Heck Hill, open bluff, 13 March 1980, *Janszen* 1532 (V 107521) and 6 Apr 1979, *Janszen* 978 (V 98035); Galiano Island, 12 May 1975, Wood 13 (V 97333); Galiano Island, west-facing slope overlooking ocean, Bluffs Park, 19 May 1993, *Benedict* 35 (UBC 207931); Gabriola Island, 21 May 1951, *Raymer* 5603135 (UBC 70999); Vancouver Island, Gonzales Hill near Victoria, April 1916, *Newcombe s.n.* (V 42590); Vancouver Island, Alberta Head, *Newcombe s.n.* (V 42592); Denman Island, wet cliffs facing Hornby Island, 7 Jul 1952, *Brink s.n.* (UBC 68843); Vancouver Island, Durrance Lake drainage on rock outcrop, 9 May 1963, *Young* 63 (UBC 108599); Vancouver Island, Ucluelet, rocky ledges, 23 May 1975, *Rose* 75-284 (UBC 177970); Vancouver Island, Anderson Hill in Victoria, 17 May 1950, *Krajina and Spilsbury s.n.* (UBC 55012); Vancouver Island, Mount Wells, 8 mi W of Victoria on moist rocky cliffs, 12 May 1975, *Calder and Taylor* 20776 (UBC 80960); Vancouver Island, Esquimalt, 17 Apr 1917, *Darling s.n.* (UBC 45840); Vancouver Island,

Victoria, 4 March 1912, *Henry s.n.* (UBC 80455); W slope of Mount Maxwell, Saltspring Island, 15 May 1963, *Young* 159 (UBC 221634); Vancouver Island, 5 km N of Cowichan Lake, 19 May 1990, *Benedict* 4 (UBC 207937); Vancouver Island, Nanoose Hill, N of Nanaimo, 1 May 1990, *Benedict* 1 (UBC 207934); Vancouver Island, Finlayson Arm Road, near Goldstream Provincial Park, 17 May 1990, *Benedict* 2 (UBC 207910); Vancouver Island, south slope of Observatory Hill, Saanich Peninsula, 1 May 1991, *Benedict* 27 (UBC 207935). USA. OREGON. **Josephine Co.**: above Rogue River 0.7 km W of entrance to Indian Mary Park, 3 May 1993, *Strayley* 7506 (UBC 208478); N of Grant's Pass near South Hill summit, 13 Apr 1991, *Benedict* 23 (UBC 208138). **Lane Co.**: S facing road cut on N side of Dorena Lake, 6 Apr 1991, *Benedict* 11 (UBC 207932); **Douglas Co.**: Umpqua River Valley, 6 Apr 1991, *Benedict* 26 (UBC 207995); Umpqua Valley, Roseburg Quadrangle, July 1914 *Cusick* 4178a, (UBC 149306); Umpqua River, 21 mi below Umpqua, 20 May 1954, *Steward* 6641, (UBC 197132). WASHINGTON. **San Juan Co.**: rock outcropping on Orcas Isl., 13 Apr 1975, *Gates* 4, (UBC 263239).

Gabriola Island, 21 May 1951, *Raymer s.n.* (UBC 5603135).

Features Distinguishing *M. sookensis* and *M. nasutus*

Mimulus sookensis is exceedingly similar in floral morphology to *M. nasutus* (Fig. 1). All characters overlap to a degree with *M. nasutus*, but under favorable growth conditions, the following structures tend to be more reduced in *M. sookensis* (*M. nasutus* measurements are presented here in parentheses): stem width <1 mm (<4 mm), calyx length 5–13 mm (6–16.5 mm), leaves $0.5\text{--}3 \times 0.5\text{--}2.5$ mm ($0.5\text{--}10 \times 0.5\text{--}7.5$ mm), height 3–25 cm (5–50 cm), pedicel length 3–22 mm (4–26 mm), stipe length 0–1 mm (0.5–2 mm). *Mimulus sookensis* tends to have a longer pistil relative to its calyx and the difference in calyx and pistil lengths range from 2.5–3.5 mm (0–6 mm). The ratio of the width of the flower to the base in *M. nasutus* is usually >2 (<2). *Mimulus nasutus* often tends to have a more sharply angled and winged stem and the leaves are often bullate, while *M. sookensis* tends to have anthocyanic red spotting on the calyx more frequently than *M. nasutus*.

Relationships and Distribution

The genus *Mimulus* contains well over 100 species of monkeyflowers, and within the Simiolus clade, there are approximately 16–24 species, including *M. guttatus*, *M. nasutus*, and *M. sookensis* (Grant 1924; Pennell 1951). Comparable to the

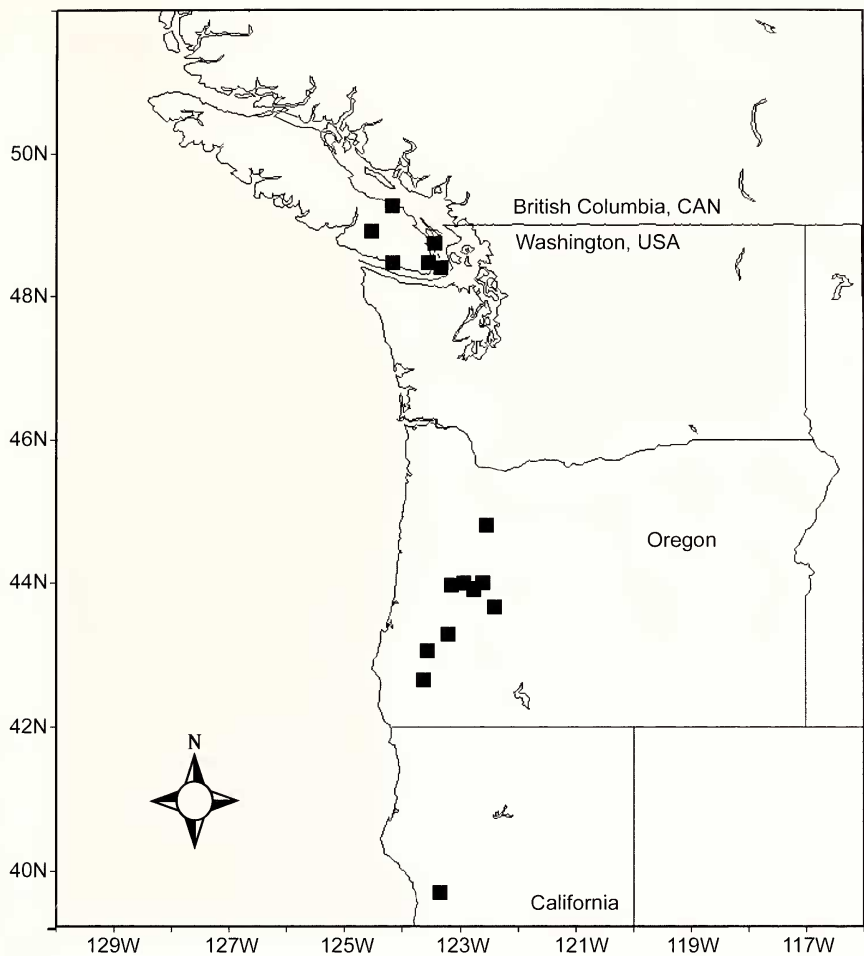


FIG. 3. Geographic distribution of *M. sookensis* in western North America, with filled squares indicating locations in which *M. sookensis* has been recorded.

rest of the genus, *M. guttatus* and its close allies are an exceedingly phenotypically and ecologically diverse group, making the *M. guttatus* complex and its close relatives an attractive system for ecological and evolutionary studies (Wu et al. 2008). Consequently, defining species relationships in this group of closely related monkeyflowers is challenging. As defined by Vickery (1978), the *M. guttatus* species complex is comprised of the common yellow monkeyflower, *M. guttatus*, and its close relatives, *M. nasutus*, *M. laciniatus* A. Gray, *M. platycalyx* Pennell, and *M. glaucescens* Greene. Pennell (1951) included a number of other taxa in the complex, including *M. nudatus* Curran, a linear-leaved serpentine endemic, and *M. pardalis* Pennell, a distinct form of monkeyflower with a prominently purple-spotted calyx, thought to be closely related to *M. nasutus* (Pennell 1947). A copper mine endemic, *M. cupriphilus* McNair, was later included in the complex (McNair 1989). Wu et al. (2008) recognize *M. guttatus*, *M. nasutus*, *M.*

laciniatus, *M. platycalyx*, *M. glaucescens*, *M. cupriphilus*, and *M. nudatus* as members of the *M. guttatus* complex at the rank of species. We suggest the addition of *M. sookensis* to this species complex.

Based on present observations, it appears that *M. sookensis* is characterized by a disjunct distribution. In the northern portion of its range, *M. sookensis* is found throughout the southern end of Vancouver Island, British Columbia, in the Gulf Islands of British Columbia, including but not limited to Saltspring, Mayne, Galiano, Denman, Lasqueti, and Pender Island, and also on the San Juan Islands of Washington (Fig. 3). In the southern portion of its range, *M. sookensis* is found in the Willamette and Umpqua River Valleys of Oregon, and also in northern California. In Oregon and California, collections are known from as far north as Mehama, in Marion Co., Oregon, and as far south as Dos Rios, in Mendocino Co., California (Fig. 3). It is conceivable that many more undiscovered *M.*

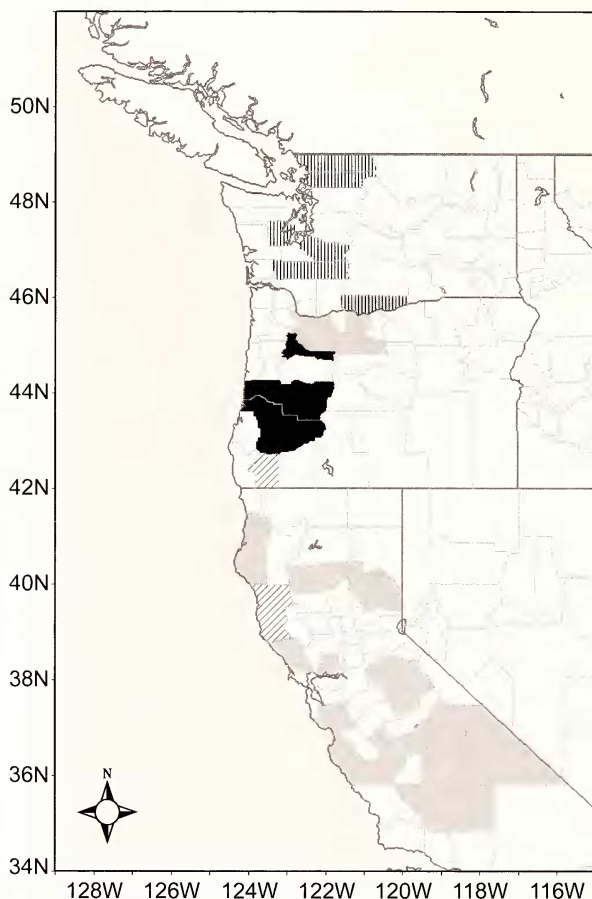


FIG. 4. Approximate location of *M. nasutus*, *M. guttatus*, and *M. sookensis* throughout western Washington, western Oregon, and California. U.S. counties where *M. sookensis* but not *M. nasutus* has been observed are filled in black, counties where *M. nasutus* but not *M. sookensis* has been observed are filled in grey, while counties where both species have been observed have diagonal hatching. Counties where *M. guttatus* has been observed in Washington are indicated with vertical hatching.

sookensis localities exist throughout the northern and southern portion of its range.

To illustrate the extent of field observations, which suggest an absence or rarity of *M. sookensis* throughout much of California, we have recorded the locations of *M. nasutus* collected in California (Fig. 4) that were used in either crossing, genetic, or flow cytometry analyses (see Table 3 and references therein). If *M. sookensis* existed further south of Dos Rios, it is likely that it would have been mistakenly collected as *M. nasutus*, and subsequent analyses would have revealed its tetraploid nature. In mainland Washington state, no *M. sookensis* have been observed to date. Kiang and Hamrick (1978) were unable to find any *M. nasutus* in the Cascades of northern California, Oregon, and Washington. Additional evidence, based on recent collections in Washington state, suggests *M. nasutus* is rare in Washington, unlike *M. guttatus* (D. Lowry, Univ. of Texas-Austin, and C. Wu, Univ. of Richmond, personal communication). At many *M. guttatus*

sites in Washington, neither *M. nasutus* nor *M. sookensis* has been observed (Fig. 4). This pattern suggests that both *M. nasutus* and *M. sookensis* may be rare in Washington state, or at the very least, that *M. guttatus* and *M. nasutus* do not commonly co-occur in this region, to our knowledge. If the rarity of co-occurrence of the two progenitor taxa in Washington state is a real phenomenon and not an artifact of sampling, the limited opportunities for hybridization between *M. guttatus* and *M. nasutus* in this region may in part explain the fact that *M. sookensis* is even more rare than *M. nasutus* in this region, and perhaps does not occur at all.

We cannot exclude the possibility that isolated or ephemeral allotetraploids derived from *M. guttatus* and *M. nasutus* are found elsewhere where *M. guttatus* and *M. nasutus* co-occur and may potentially hybridize. However, determining the exact range limits of *M. sookensis* is beyond the scope of this paper, and we present here simply what is known at this time regarding the

TABLE 3. LIST OF LOCALES USED TO ILLUSTRATE LOCATIONS OF *M. NASUTUS*, *M. SOOKENSIS*, AND *M. GUTTATUS*, THAT HAVE BEEN CONFIRMED TO BE OF DIPLOID OR TETRAPLOID NATURE, THROUGH EITHER GENETIC ANALYSES, CROSSING EXPERIMENTS, CHROMOSOME COUNTS, OR FLOW CYTOMETRY. The locale ID may refer to: 1) the culture number given in a published chromosome count, 2) an examined herbarium specimen accession number or collector number, or 3) the ID given to the locale when published. Abbreviations used: na = not applicable.

Species	Locale ID	County, State	Reference
<i>M. nasutus</i>	16	Calaveras Co., CA	Benedict 1993
	KIN	Fresno Co., CA	Sweigart and Willis 2003; Sweigart et al. 2007
	SNF	Fresno Co., CA	Sweigart and Willis 2003
	na	Fresno Co., CA	Kiang and Hamrick 1978
	KNR	Humboldt Co., CA	Modliszewski and Willis (unpublished data)
	Cult. No. 6060	Inyo Co., CA	Mia et al. 1964
	na	Kern Co., CA	Kiang and Hamrick 1978
	BRI	Mariposa Co., CA	Sweigart and Willis 2003; Sweigart et al. 2007
	na	Mariposa Co., CA	Kiang and Hamrick 1978
	NDR2	Mendocino Co., CA	Sweigart and Willis 2003; Martin and Willis 2010
	SHI	Mendocino Co., CA	Modliszewski and Willis (unpublished data)
	Cult. No. 5044	Monterey Co., CA	Vickery 1955
	na	Plumas Co., CA	Kiang and Hamrick 1978
	MHA	Santa Clara Co., CA	Modliszewski and Willis (unpublished data)
	Cult. No. 5751	Santa Clara Co., CA	Vickery 1964
	NBC	Santa Cruz Co., CA	Sweigart and Willis 2003
	na	Sierra Co., CA	Kiang and Hamrick 1978
	NMD	Solano Co., CA	Sweigart and Willis 2003
	CMF	Sonoma Co., CA	Modliszewski and Willis (unpublished data)
	KRR	Sonoma Co., CA	Modliszewski and Willis (unpublished data)
	Cult. No. 5865	Sonoma Co., CA	McArthur et al. 1972
	M12	Tehama Co., CA	Sweigart and Willis 2003; Sweigart et al. 2007
	TOK	Tulare Co., CA	Sweigart and Willis 2003
	na	Tulare Co., CA	Kiang and Hamrick 1978
	Cult. No. 5327	Tuolumne Co., CA	Mukherjee and Vickery 1962; n = 13
	NDP	Tuolumne Co., CA	Sweigart and Willis 2003; Martin and Willis 2010
	MEN	Tuolumne Co., CA	Sweigart and Willis 2003; Martin and Willis 2010
	NCL	Tuolumne Co., CA	Sweigart and Willis 2003; Sweigart et al. 2007
	NFN	Clackamas Co., OR	Modliszewski and Willis (unpublished data)
	HCN	Josephine Co., OR	Modliszewski and Willis (unpublished data)
	TRT	Multnomah Co., OR	See text
	SF	Wasco Co., OR	Fishman and Willis 2001; Sweigart and Willis 2003; Martin and Willis 2010
	WSK	Klickitat Co., WA	Modliszewski and Willis (unpublished data)
	CLR	Klickitat Co., WA	Sweigart and Willis 2003; Sweigart et al. 2007
<i>M. sookensis</i>	BVN	Douglas Co., OR	Fig. 3
	WBP	Douglas Co., OR	Fig. 3
	Benedict 207995	Douglas Co., OR	See text
	ROG	Josephine Co., OR	See text; Sweigart et al. 2008
	Straley 208478	Josephine Co., OR	See text
	Benedict 208138	Josephine Co., OR	See text
	DRN	Lane Co., OR	Sweigart et al. 2008
	HIL	Lane Co., OR	Fig. 3
	LSN	Lane Co., OR	See text; Sweigart et al. 2008
	PSG	Lane Co., OR	Sweigart and Willis 2003; Sweigart et al. 2008
	SPB	Lane Co., OR	Sweigart et al. 2008
	Benedict 207932	Lane Co., OR	See text
	SAN	Marion Co., OR	Fig. 3
	WTU 263239	San Juan Co., WA	See text
	NDR	Mendocino Co., CA	Sweigart and Willis 2003; Sweigart et al. 2008
<i>M. guttatus</i>	WSKG	Klickitat Co., WA	Modliszewski and Willis (unpublished data)
	RFA	Lewis Co., WA	Modliszewski and Willis (unpublished data)
	HAM	Mason Co., WA	C. Wu, personal communication
	HOC	Mason Co., WA	D. Lowry, personal communication
	CHR	Pierce Co., WA	C. Wu, personal communication
	AWP	Skagit Co., WA	Modliszewski and Willis (unpublished data)
	NCG	Whatcom Co., WA	Modliszewski and Willis (unpublished data)

distribution of *M. sookensis* based on current collections.

Interestingly, while *M. sookensis* commonly co-occurs with *M. guttatus* throughout its range, with few exceptions, in habitats where *M. sookensis* is present, *M. nasutus* tends to be absent. *Mimulus nasutus* and *M. sookensis* are known to co-occur at only two locations. Although Vancouver Island is at the northern limit of the range of *M. nasutus*, it is found to co-occur with *M. sookensis* at one site on the southern end of Vancouver Island (Nanoose Hill). This site is at a lower elevation than many of the other locations on Vancouver Island where only *M. sookensis* was observed (Fig. 3, Benedict 1993). The second site is along the Rogue River in southern Oregon; other *M. nasutus* sites have also been found in this region (Table 3). Additionally, at the southern periphery of the range of *M. sookensis* near Dos Rios, California, *M. nasutus* and *M. sookensis* are found within ca. 3 km of one another, but not within the same collection locale (Sweigart and Willis 2003). At present, there is insufficient evidence to determine whether or not the apparent absence of *M. nasutus* at many of the *M. sookensis* collection locales is a historical artifact or if the relative rarity of co-occurrence is caused by some unknown biological or abiological factor.

DISCUSSION

Within just the *Simiolus* clade of the genus *Mimulus*, there are over 21 well-documented occurrences of polyploidy or aneuploidy (reviewed in Beardsley et al. 2004). The *Mimulus glabratus* heteroploid species complex in the *Simiolus* clade is characterized by ploidal races that are distributed across a north-south latitudinal gradient (McArthur et al. 1972). Crossing barriers exist both between ploidal races, and to varying extents, within ploidal races (Alam and Vickery 1973; Vickery et al. 1976).

Here, together with data from previous publications (Fig. 2; Table 2; Sweigart et al. 2008), we have presented evidence of another instance of polyploid speciation—the previously undescribed *M. sookensis*. Although the triploid block is not absolutely complete between *M. sookensis* and its diploid progenitors, a triploid bridge is not likely to contribute significantly to gene flow or polyploid formation in a selfing taxa (Ramsey and Schemske 1998). Vickery found many other forms of polyploid and aneuploid monkeyflowers in the *M. guttatus* species complex during the course of his extensive cytogenetic work in *Mimulus*, but no record exists of *M. sookensis* (Mukherjee and Vickery 1959, 1960, 1962; Mia et al. 1964; Mia and Vickery 1968; Vickery et al. 1968; McArthur et al. 1972). Most of the autotetraploid *M. guttatus* that Vickery found were in the southwestern U.S. (Arizona, Colorado, New Mexico,

and Utah) and Mexico or in Alaska, but one autotetraploid *M. guttatus* was found in Multnomah Falls, near Portland, Oregon. This individual was likely not *M. sookensis*, since Vickery's identification indicates that it bore more resemblance to *M. guttatus* than *M. nasutus*. Within the *M. guttatus* species complex, the autotetraploid *M. guttatus* subsp. *haidensis* Calder and Taylor is a distinct form of *M. guttatus* endemic to the Haida Gwaii (Queen Charlotte Islands) of British Columbia, Canada. Despite these autotetraploid forms of *M. guttatus*, *M. sookensis* will continue to remain a distinct species, due to the fact that the progeny of a cross between autotetraploids and allotetraploids will be tetraploid, and any backcrossing with a diploid will occur in the direction of the outcrossing species (*M. guttatus*), not the selfing species (*M. sookensis*). These backcross progeny, if existent, will likely be inviable or infertile, as was shown in Sweigart et al. (2008). Additionally, data from nuclear genes (Sweigart et al. 2008) does not show loss of *M. nasutus* gene copies, which would be expected if hybridization with autotetraploid *M. guttatus* had occurred.

The newly described *M. sookensis* is broadly distributed in scattered locations throughout the valleys of western Oregon and northern California, and also on the southern tip of Vancouver Island and the Gulf Islands of British Columbia and San Juan Islands of Washington. The seemingly disjunct distribution of *M. sookensis* raises the question as to whether or not the distribution is actually discontinuous, or if *M. sookensis* exists undiscovered in Washington; further field work in Washington could help to determine if the observed distribution is real. Data from plants of the Pacific Northwest suggest that the glaciations of the Pleistocene created discontinuous distributions that were later recolonized (Soltis et al. 1997). If *M. sookensis* formed post-Pleistocene glaciation events, it may be that *M. nasutus* has yet to extensively recolonize Washington state, in contrast to the more common *M. guttatus*, and that the rarity of *M. nasutus* in Washington has contributed to more extreme rarity of *M. sookensis* in Washington. If *M. sookensis* formed throughout the Pacific Northwest prior to Pleistocene glaciations, it may have existed in glacial refugia on Vancouver Island and Oregon (Soltis et al. 1997; Brunsfield et al. 2001; Shafer et al. 2010), and has not yet extensively recolonized Washington.

Of final note is the observation that *Mimulus sookensis* from different collection locations all appear to be phenotypically quite similar to *M. nasutus*. It would be interesting to know if *M. sookensis* was formed by multiple polyploidization events, as suggested by sequences from one of two nuclear genes sequenced to date (Sweigart et al. 2008), or if individuals from as far apart as British Columbia and California originated once,

and then spread geographically to occupy their current distribution. If *M. sookensis* was indeed formed by multiple allopolyploidization events, as is common among polyploid plants (Soltis and Soltis 1993, 1999) it would be of great interest to know how these interspecific polyploid hybrids between *M. guttatus* and *M. nasutus* all came to have the appearance of *M. nasutus*.

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